

Lack of association between the G-660C polymorphism in the dopamine transporter gene (*SLC6A3*) and schizophrenia in the Iranian population

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BACKGROUND: Dopaminergic system plays an essential role in the plasticity of the human brain. The dopamine transporter gene (*SLC6A3*) mediates active reuptake of dopamine from synapses, terminates dopamine signals, and therefore, is implicated in a number of dopamine-related disorders like psychosis. Variations in the form of single nucleotide polymorphisms in the core promoter of the *SLC6A3* gene are reported to be involved in the pathogenesis of schizophrenia. In this study, we also attempted to establish the possible role of the polymorphism G-660C in the *SLC6A3* gene promoter in schizophrenia in a case-control study.

MATERIALS AND METHODS: The allele and genotype frequency were analyzed in an Iranian cohort of 200 unrelated patients and 200 controls using polymerase chain reaction and restriction fragment length polymorphism.

RESULTS: The genotype frequency for case and control groups was GG 100%, GC 0%, CC 0%, and GG 100%, GC 0%, CC 0%, respectively. The C allele was failed in both groups.

CONCLUSION: Our data suggest clearly that there is no association between the -660G/C polymorphism and outcome of schizophrenia in the Iranian population.

Key words: Schizophrenia, dopamine transporter gene, polymorphism, Iranian population, association study

neuronal network in the human brain and is involved in the control of motor function, cognition, and behavior.^[1-2] The human dopamine transporter (*SLC6A3*) is a member of the sodium and chloride-dependent neurotransmitter transporter family and contains 12 transmembrane domains.^[3,4-8] The dopamine transporter terminates the synaptic transmission by the rapid and specific reuptake of dopamine into the presynaptic nerve terminals.^[1,9-12] Psychostimulants, such as cocaine and amphetamine, are known to bind the dopamine transporter and inhibit dopamine reuptake.^[13] In DAT knockout mice, dopamine clearance is prolonged and the mice show spontaneous hyperactivity.^[14-18] The reduction in dopamine reuptake results in adaptive changes such as a decreased content of dopamine in presynaptic terminals and significant downregulation of D1 and D2 receptor expression.^[6]

The dopamine hypothesis of psychosis is a model attributing symptoms of schizophrenia to a disturbed and hyperactive dopaminergic signal transduction.^[7,8] However, several reports suggested strong association between *SLC6A3* gene variants and schizophrenia.^[6,9] It has further been shown that the most variants in this gene are located within the putative binding site for transcription factors. The allelic variants would also impact the nature of these response elements.^[19-22] To date, some single nucleotide polymorphisms have been identified in the core promoter of the *SLC6A3* gene, which appear to be linked with the outcome of schizophrenia. One of these variants is the T-67A polymorphism, and has previously been investigated by Khodayari *et al.* in a set of Iranian subjects and controls. Their findings provided tentative

Introduction

Dopamine is the major neurotransmitter of the

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.100773

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evidence for the contribution of *SLC6A3* gene core promoter polymorphism to the etiology of schizophrenia in the Iranian population.^[20] We attempted also to investigate in a case-control study, the relevance of the G-660C polymorphism, in the Iranian population.

Materials and Methods

Subject

For the case-control study, 200 unrelated patients with a mean age of 43.34 (SD=11.353) were recruited from hospitals in south and south-west of Iran. Of these, 117 were men (63 from south and 54 from south-west) and 83 were women (48 from south and 35 from south-west). The diagnosis of schizophrenia was based on DSM IV criteria. The control group consisted of 200 healthy blood donors with a mean age of 39.43 (SD=11.103), which were matched for gender and ethnicity to the patient cohort. An informed consent was obtained from all participants.

Genotyping

Genomic DNA was extracted from whole blood using the standard salting out method. The G-660C polymorphism in the *SLC6A3* gene was screened using the PCR-RFLP method. Sequence of primers used to amplify 260 nucleotides from 5'-flanking region of *SLC6A3*, contains -660G/C polymorphism were F: 5'-TGGAGCCGGATACCAACC-3', and R: 5'-TCCACCTCTGACCAGC-3'. Following a melting step at 94°C for 5 minutes, 35 cycles of 94°C for 30

seconds, 60°C for 30 seconds and 72°C for 30 seconds was used for amplification and a final elongation step of 72°C for 5 minutes. PCR products were digested overnight at 37°C with 10 units of restriction endonuclease HhaI (*Biolabs*), which cuts only the G allele, and were analyzed by gel electrophoresis.

Statistical analysis

The Chi-square test was performed to compare genotypes within and between genders. Chi-square tests were calculated using the SPSS 13 statistical package.

Results

The study of socio-demographic features of the cohort identified that subjects in the case group were significantly lower than those in the control group in terms of marital status and educational level ($P < 0.05$). Control and patient groups were initially classified only by gender and were not statistically different in terms of their socio-demographic features ($P > 0.05$) [Table 1]. The genotypic frequencies of individuals from the 2 geographical regions (south and south-west Iran) were GG 100%, GC 0%, CC 0%, respectively [Table 2]. However, no C allele has been seen among the case-control groups.

Discussion

The relevance of genetic factors in the etiology of schizophrenia has been demonstrated in many studies,^[3] and the hypoexpression of *SLC6A3* has been

Table 1: Socio-demographic characteristics of the case and controls

Variables	Cases	Controls
Continuous variable		
Age	43.34±11.353	39.43±11.103
Age of onset	22.01±9.047	-
Discontinuous variables		
Sex		
Females	83	83
Males	117	117
Educational level		
Illiteracy	28	3
Primary school	113	13
High school	42	145
College	7	39
Marital status		
Single	139	43
Married	35	156
Divorced	16	1

Table 2: Allelic frequencies and genotypic distribution of the -660G/C polymorphism in the DAT1 gene

Allele and genotype frequency	Cases	Controls
Allelic frequencies		
South		
G	1.00	1.00
C	0.00	0.00
South-west		
G	1.00	1.00
C	0.00	0.00
Genotypic distributions		
South		
GG	100	100
CG	0.00	0.00
CC	0.00	0.00
South-west		
GG	100	100
CG	0.00	0.00
CC	0.00	0.00

hypothesized as a possible etiology of schizophrenia.^[6] Dysregulation of hSLC6A3 expression could be related to a particular contribution of polymorphisms across the gene.^[20-22] The *SLC6A3* promoter region displayed 5 polymorphic sites: T-67A, G-660C, C-839T, C-1169G, and T-1476G. They are located within a core region that regulates the activity of luciferase construct with 10-150 times more activity than promoter less construct. Computational analysis of G-660C SNP revealed that this polymorphism is located within a putative Sp1-binding site, and -660C introduces a LF-A1 binding site. Whereas Sp1-binding site are ubiquitously expressed in mammalian cells, the transcription factor LF-A1 interacts with the promoter region of several genes expressed in hepatocytes.^[22] Indeed, the functional data about the mentioned SNPs make them attractive to be involved in the etiology of schizophrenia and so far association studies. However, in 2004, Khodayari *et al.* carried out an association study in a cohort of Iranian subjects and controls. They have found an association between the *SLC6A3* gene core promoter polymorphism -67A/T and schizophrenia.^[20] In the present survey, the polymorphism -660G/C was proved in a case/control study in a relatively large size of samples. Our results revealed lack of association between this polymorphism and schizophrenia disorder. Studies about European populations showed similar results.^[21,22] But, in contrast to the previous reports, the present study indicates definitely the lack of C allele in 400 individuals of population from south and south-west of Iran. In 800 screened alleles, the G allele was the only one that was observed. The highlight of our results is also the absence of C allele in the gene pool of Iranian population that we studied. Therefore, this polymorphism may not have any role in pathophysiology of schizophrenia, and the -660G/C variant of *SLC6A3* gene seems to be not proper candidate for etiology of schizophrenia, at least in Iranian patients.

Acknowledgment

This study was supported in part by grant of Shahid Chamran University of Ahwaz, and we thank from Ahwaz and Shiraz hospitals for collaboration and preparation of samples in this study.

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Cite this article as: Foroughmand AM, Galehdari H, Dastgerdi BT, Khatami SR, Haidari M. Lack of association between the G-660C polymorphism in the dopamine transporter gene (*SLC6A3*) and schizophrenia in the Iranian population. *Indian J Hum Genet* 2012;18:222-5.

Source of Support: supported in part by grant of Shahid Chamran University of Ahwaz, **Conflict of Interest:** None declared.

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